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Received October 28, 1966. P.S.E.B.M., 1967, v124.

Insulin Secretion by Isolated Islets in Presence of Glucose, Insulin and Anti-Insulin Serum. (31773)

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Two methods have recently been introduced for measurement of insulin secretion *in vitro*. In one(1), small pieces of pancreas from the rat are incubated with guinea pig anti-insulin serum (GPAIS) which serves two purposes. The antibodies to bovine insulin combine with the secreted hormone before it can be destroyed by lytic substances released from the acinar tissues, and their neutralization during incubation gives a measure of the rate at which insulin is secreted. In the second method(2) individual Islets are isolated after treatment of the rat pancreas with collagenase. Secreted insulin, which is not then exposed to the lytic effects of any acinar tissue, accumulates in the incubation medium and can be measured by conventional methods of immuno assay. By using a combination of these techniques, it is here shown that the insulin content of an incubation medium has no effect upon insulin secretion by isolated Islets of Langerhans.

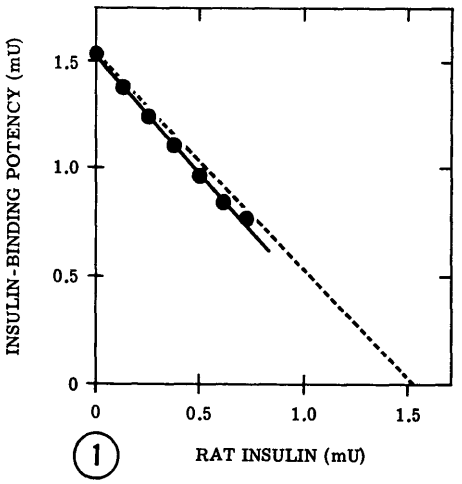
Materials and methods. Isolated Islets were obtained from the pancreas of the normal rat by the method described by Lacy and Kostianovsky(2) and incubated (5 or 10 islets/1.0 ml) in a bicarbonate-buffered medium equilibrated with oxygen (95%) and carbon dioxide (5%) and containing bovine serum albumin (0.5%, w/v; bovine albumin, Fraction V, Sigma Chemical Co., St. Louis, Mo.). To this medium, as required, were added glucose (30, 150 or 300 mg/100 ml), rat insulin, and GPAIS. The rat insulin (19.5 U/mg; Lot No. R 564; Novo Research Institute, Copenhagen) was dissolved in dilute acetic acid (0.6%, v/v, glacial) to give a stock solution (100 mU/ml) which was stored in the frozen state. The same batch of guinea pig anti-insulin serum (Lot 270) was used throughout. A stock solution (8%, v/v) was prepared in phosphate buffer (0.1 M; pH 7.0) containing bovine albumin (1%, w/v) and stored in the frozen state; no change in insulin binding potency of this diluted GPAIS (86.4 $\pm$ 0.4 mU bovine insulin/ml, n = 7) was observed over the short period (7 days) needed to complete the present studies.

To study *destruction of added insulin* by pancreatic tissue, constant amounts of unlabeled (540 μU/ml, PJ 4600, Eli Lilly and Co., Indianapolis) and ^{131}I-labeled (60 μU/ml; 9.6 mc/mg; In 281-2, Abbott Laboratories, North Chicago, Ill.) bovine insulin

were incubated in the presence of isolated islets (10 islets/ml) or a similar number of small pieces of acinar tissue. At varying times up to 90 minutes later, aliquots (0.8 ml) of media were removed and added to a suspension of finely divided cellulose (10%, w/v; MN 300, Macherey, Nagel and Co., Duren, Germany) in phosphate buffer (0.1 M, pH 7.0). Thirty minutes later and after centrifugation, the radioactive contents of aliquots of supernatant solutions were measured. The radioactive contents of these solutions, for reasons given elsewhere(3), were assumed to be proportional to their contents of denatured or damaged insulin and are expressed as a percentage of the total added radioactivity.

To measure *insulin secretion,* groups of 5 Islets were first incubated for 30 minutes in a medium (1.0 ml) containing only glucose (100 mg/100 ml) and then for 60 minutes in a separate medium (1.0 ml) containing, as required, glucose, rat insulin or GPAIS. When GPAIS was present in the medium, unneutralized antibodies were directly assayed in an aliquot (0.6 ml) of the incubated medium. Otherwise, an aliquot (0.4 or 0.5 ml) of that medium was first allowed to react for 30 minutes at 37°C with a constant volume (0.2 or 0.5 ml) of appropriately diluted GPAIS, and then assayed for unneutralized insulin antibodies. The method of assay has been described in detail(1,3). Under the conditions here used, and as shown in Fig. 1, unneutralized antibodies measured during the assay are linearly related to the amount of rat insulin initially present in the samples; a more detailed account of these reactions will be published elsewhere. Insulin secreted (mean ± SEM) from 5 islets into the medium (1.0 ml) during incubation is equated to the decrease in reactive antibody content of that medium.

Results. The lytic effects of isolated islets, small pieces of acinar tissue, and of media from which the latter had been removed are summarized in Fig. 1. They show that like media incubated for similar periods in the absence of any tissue, media containing isolated islets have no lytic effect upon added insulin during 90 minutes. On the other hand, media containing small pieces of acinar tissue, and to a lesser extent media from which such tissues had been removed after 45 minutes incubation, destroyed added bovine insulin at a rapid rate.



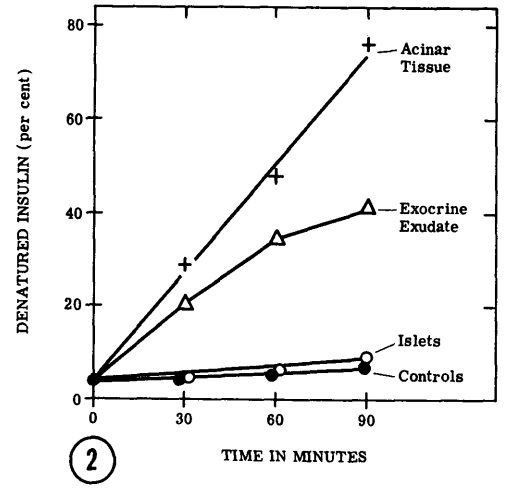


FIG. 1. Insulin binding potencies of constant volumes (1.35 μl) of GPAIS (Lot 270) after incubation for 60 minutes at 36°C with increasing amounts of rat insulin. Insulin binding (ordinate) is quoted as mUnits bovine bound by sera after treatment with rat insulin (abscissa). The theoretical relationship is shown as a dotted line.

FIG. 2. Percentages of added insulin denatured during 90 minutes by media containing no incubated tissue (Controls), isolated Islets (Islets), and comparable amounts of isolated acinar tissue (Acinar tissue); and by medium previously incubated with acinar tissue but from which this tissue had been removed (Exocrine exudate). Each point represents the mean of 5 to 9 individual observations.

TABLE I. Mean Insulin Output (± SEM) by 5 Islets Incubated in Absence (Control) or Presence of GPAIS. Number of observations (in parentheses) and significances of differences in secretion rate (P) are given for each glucose concentration.

Glucose (mg/100 ml)	Secreted insulin (μU/5 islets/60 min) Control	GPAIS	P
30	62 ± 7 (9)	58 ± 9 (9)	>0.7
150	376 ± 22 (9)	356 ± 22 (9)	>0.6
300	800 ± 68 (12)	860 ± 82 (12)	>0.5

Since isolated islets do not destroy added insulin, they were incubated under various experimental conditions to assess the effects of GPAIS and insulin upon secretion.

1. *Effect of added GPAIS on secretion.* As shown in Table I insulin secretion rose as the glucose content of the medium was increased from 30 to 300 mg/100 ml. The amount of insulin found in media to which no GPAIS was added until the incubation was complete was the same as that secreted into media to which GPAIS was added at the outset.

2. *Effect of added insulin on secretion.* When rat insulin (600-1,000 μU) was added at the beginning of incubation for 60 minutes, and the GPAIS at the end, there were no significant differences in insulin secretion rates at 3 concentrations of glucose from those observed in the absence of added insulin (Table II).

Discussion. The present results demonstrate that neither insulin nor GPAIS have a direct effect upon insulin secretion. That insulin could have such an effect was suggested by Hausberger and Ramsay(4), who found a less marked degranulation of the beta cells after simultaneous administration of glucose and insulin to guinea pigs than after glucose alone. However, these preliminary observations were not confirmed by Logothetopoulos *et al*(5). More recently, Frerichs *et al*(6), using pancreatic tissue of the rat, produced evidence to suggest that insulin added to an incubation medium could inhibit secretion. However, they took little account of the lytic effect of such tissue which has a marked effect on incubated insulin(1); they also used a bioassay for the measurement of insulin.

More suggestive evidence has been provided by Logothetopoulos, *et al*(7) who showed that degranulation of the beta cells and pancreatic insulin depletion over 5 hours were much more marked in rats injected with GPAIS than in rats maintained at a comparable level of hyperglycemia with infused glucose. They tentatively concluded that this difference could be due to the creation by GPAIS of a steep gradient of insulin concentration across the membranes separating the blood containing no free insulin, and the beta cells with their large stores of hormone. In view of the present result this explanation is unlikely to be correct and some other factors must be responsible for the rapid depletion of the granules in the pancreas of the rat injected with GPAIS. As pointed out by Logothetopoulos *et al* themselves, the insulin deficiency due to GPAIS leads not only to hyperglycemia, but to other metabolic changes which are quite different from those induced by glucose infusion of the normal animals. The effect of such metabolic (and possibly hormonal) changes on insulin

TABLE II. Mean Insulin Output (± SEM) by 5 Islets Incubated in Presence or Absence of Rat Insulin. Amount of secreted insulin is calculated as the difference between insulin contents of media before (added) and after (recovered) incubation. Also shown here is the significance of differences in secretion rate in presence or absence of insulin, at the 3 glucose concentrations.

Glucose (mg/100 ml)	Insulin (μU/5 islets/60 min) Recovered after incubation	— Added before incubation	= Secreted	P
30	164 ± 37 (7)	0	164 ± 37	>0.4
	716 ± 34 (7)	596 ± 31 (3)	120 ± 47	
150	321 ± 34 (9)	0	321 ± 34	>0.9
	942 ± 43 (9)	622 ± 19 (3)	320 ± 77	
300	700 ± 93 (9)	0	700 ± 93	>0.4
	1664 ± 38 (9)	1064 ± 57 (3)	600 ± 73	

secretion remains to be investigated.

The present findings are not incompatible with the effects observed after repeated administration of exogenous insulin *in vivo*. Several investigators have reported a decline in the insulin content of the pancreas(8,9). In such animals, hypoglycemia has been reported, and this could explain the reduced insulin content of the pancreas, for Parry and Taylor(10) have recently shown that insulin synthesis in isolated pancreatic tissue is related directly to glucose concentration in the incubation medium.

Summary. Isolated Islets of Langerhans from the rat's pancreas were incubated in media containing varying combinations of glucose, rat insulin, and guinea pig anti-insulin serum (GPAIS). Neither insulin nor GPAIS modified the rate at which insulin was secreted in response to glucose. The concept that insulin in the fluids surrounding Islet tissue can inhibit secretion of insulin by the β-cells is discussed in the light of these results.

The authors wish to acknowledge grants in aid of this research (PHS AM 07211-03, AM 06181, AM 03373), and for a Post-doctoral Fellowship (1 FO 5-TW-865-01) for one of us (W.M.). Our thanks are also due to Dr. I. Schlichtkrull (Novo Research Institute, Copenhagen, Denmark) and the European pool of insulin for a generous supply of rat insulin; and to Mr. Donald Young and Mrs. Jean Fink for skilled technical assistance.

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Received October 12, 1966. P.S.E.B.M., 1967, v124.

New Metabolizable Immunologic Adjuvant* for Human Use. 6. Disposition of Radioactivity after Administration of Labelled Vaccine To the Rat. (31774)

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Previously, it was shown(1-3) that emulsions prepared with Adjuvant 65 were almost as effective immunologically as preparations which contained mineral oil, but were considerably less irritating. Since all of the components of the adjuvant are forms of normally occurring dietary substances, they should be either metabolized or excreted by the animal organism. Histologically, it was shown that the initial deposits in the muscle became diffusely encapsulated and gradually disappeared. However, there was no information on the manner in which the components were disposed of after injection as the unique adjuvant-vaccine formulation. Consequently, 3 different preparations, each containing one of the adjuvant substituents labelled with carbon-14, were administered to rats. From radio counts of the injection sites, carcasses and excreta, it is concluded that the adjuvant is absorbed, and its components are metabolized or excreted.

Materials. Aluminum monostearate carboxy-C^{14} was supplied by Dr. C. Rosenblum, Merck Sharp & Dohme Research Laboratories, Rahway, N. J. It had a melting point of 190-194°, and the ash content,

* Adjuvant 65.